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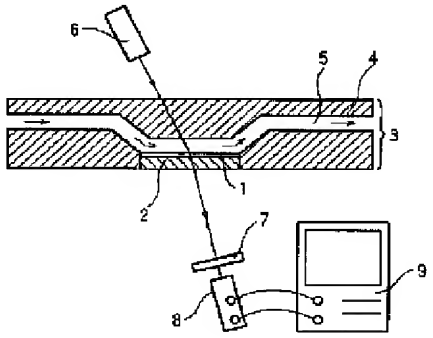
(54) MOLECULE RECOGNIZING FUNCTION FILM AND SENSOR EMPLOYING IT

(57) Abstract:

PURPOSE: To obtain a molecule recognizing function film, and a sensor employing the film, for detecting variation of concentration of specific molecule by converting into variation of secondary harmonic generating power.

CONSTITUTION: A molecule recognizing function film 1 is formed on a transparent substrate 2 and disposed such that a sample 5 flowing through a channel 4 in a transparent flow cell 3 touches the film 1 directly. Light (basic wave) emitted from a light emitting element 6 transmits through the flow cell 3 and the sample channel 4 and impinges on the film 1. Light transmitted through the film 1 enters into a cut filter 7 where wavelengths other than second harmonic are cut off and only second harmonic impinges on a light receiving element 8. Variation of secondary harmonic is detected and processed at a signal processing section 9 thus determining concentration or specific element contained in the sample 5.



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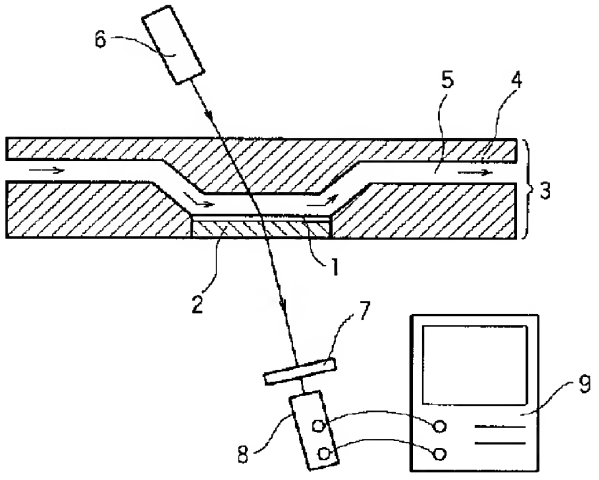
(54)【発明の名称】　分子認識機能膜及びこれを用いたセンサー

(57)【要約】

【目的】　特定分子の濃度変化を二次高調波発生強度の変化に転換して検出する分子識別機能性膜及びこれを用いたセンサーを提供する。

【構成】　分子認識機能膜１を透明基板２上に形成し、透明なフローセル３内の流路４を流れる測定試料５が直接分子認識機能膜１に触れるように設置する。発光素子６から発せられた光（基本波）は、フローセル３及び測定試料５の流れる流路４を透過して分子認識機能膜１を照射する。分子認識機能膜１を透過した二次高調波を含む光は、カットフィルター７で二次高調波以外の波長をカットされて受光素子８へ入射する。検出した二次高調波の変化を信号処理部９で処理して測定試料５中に含まれる特定試料の濃度を算出する。



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【特許請求の範囲】

【請求項１】　特定化合物との酵素反応等の触媒機能、この化合物との吸着機能、或いはこの化合物との錯体形成機能等を有する分子認識物質を担体に固定化した分子認識機能膜において、前記膜が単分子膜を非対称構造に重ねた累積膜であり、この累積膜を構成する複数の単分子膜の少なくとも一つには、二次高調波を発生する化合物が含有されていることを特徴とする分子認識機能膜。

【請求項２】　前記二次高調波を発生する化合物が、電子供与基及び電子受容基に挟まれた発色団を有する化合物である請求項１に記載の分子認識機能膜。

【請求項３】　前記分子認識機能膜が、親水基と疎水基とを有する化合物を含む一種以上の単分子膜を重ねた累積膜である請求項１に記載の分子認識機能膜。

【請求項４】　単分子膜を非対称構造に重ねた累積膜であって、この累積膜を構成する複数の単分子膜の少なくとも一つに、二次高調波を発生する化合物が含有されている分子認識機能膜と、基本波を発生して前記分子認識機能膜へ入射する発光素子と、ここから発生する二次高調波を検知する受光素子と、受光素子からの信号を処理する信号処理部からなるセンサー。

【発明の詳細な説明】

【０００１】

【産業上の利用分野】本発明は、有機及び無機化合物或いは生体関連化合物等の濃度測定を二次高調波発生によって行う分子認識機能膜及びこの膜を使用したセンサーに関する。

【０００２】

【従来の技術】生体は無機物や有機物を高い選択性で認識する能力を有している。その高い選択性を有する機能性分子、或いは細胞、組織を始め生体そのものを物質選択機能部位として、試料溶液中の検体を迅速に簡単に検出するバイオセンサーが知られている。

【０００３】上記センサーの機能は、特定の検体を選択的に認識して反応させる部分と、この反応による検体の導伝性、発熱、発光等の変化を捉らえて信号に変換する部分に分けて考える事ができる。検体を認識してこれと反応する生体高分子としては、酵素、抗体、受容体等の蛋白質などが知られており、これらは天然高分子や合成高分子から成る膜中に分散・固定化して用いられる。一方、反応を捉らえて信号に変換する部分には、一般的に酸素電極、過酸化水素電極、イオン電極、ガス電極などの電極が用いられている。また最近は上記検体の発光を利用したセンサー等が数多く提案されてきている。

【０００４】前記の電極を用いる電気化学的測定法は、(1)測定に際して電気的、磁気的ノイズが発生しやすいこと、(2)参照電極を用いるために微量の検体を測定できないこと、等の難点がある。これに対して光学的測定方法は例えば特開昭61-292045、特開昭63-75542、特開昭63-271162の各号公報に開示されているが、(1)近年の

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技術革新により高感度の光検出が可能になったこと、(2)測定に際して電気的、磁気的ノイズが発生しにくいこと、(3)参照電極は不要なこと、等の利点があり、更に光の入射や検知に光ファイバーを用いた場合には、反応場と光ファイバーとを物理的に接触させる必要がないため、反応場の試薬交換が容易となること、また、複数の波長を用いて複数の検体を同時に測定できる可能性があること等の特徴を有している。

【０００５】一方前記の二次高調波発生用の材料として、有機非線形光学材料は原理的に無機材料よりも高い非線形光学特性を実現できる可能性があるため精力的に研究されている。高い二次高調波発生（ＳＨＧ）を実現させるためには、分子分極率の高い分子を、反転中心を持たない配列構造（非対称配向）に配列させることが必要である。そのため当初は単結晶材料に関して進められてきた研究が、近年ではラングミュアーブロジェット（ＬＢ）法によって形成される非対称配向構造を有する累積膜についても行われるようになってきている（応用物理　第60巻　第6号(1991) P586）。このＬＢ法は、(1)反転中心を持たない配列構造（非対称配向）が容易に形成できること、(2)常に基板の法線方向に光学軸を有する薄膜として得られること、(3)分子レベル単位で薄膜を制御できること、(4)重合性基を導入することにより、リソグラフィ技術を用いてパターニングが可能なこと、(5)重金属等の物質を導入して屈折率を制御できること、といった特徴を有しているが、更に、成膜成分として分子分極率の高い分子を用いると、形成されるＬＢ膜自身も高い分極特性を示し、従って高い二次高調波発生を示すという大きな特徴がある。

【０００６】上記の二次高調波発生は非線形光学材料の特性の一つであるが、ＬＢ膜の分子配列或いは分子構造に変化があると非線形光学特性も影響を受ける。この原理を応用して塩化水素ガスでＬＢ膜成膜成分のプロトン化反応を行い、このときの二次高調波変化を分析した例がある（電気化学会　'91春年会 2A22）。

【０００７】

【発明が解決しようとする課題】上述した従来のＬＢ法は二次高調波を発生することができるが、センサーとしてどのように利用するかについては未だ研究の段階にあり、実用に供されていない。上記分子構造の変化と非線形光学特性とを結びつけた研究にあっても、この技術はＬＢ膜構成分子が直接的な化学変化を受けるため、分析機器として使うには不向きであった。

【０００８】

【課題を解決するための手段】上記課題を解決すべく本願発明は、単分子膜の成膜成分中に分子認識機能を有する化合物及び二次高調波を発生する化合物を導入して累積膜を形成する。従ってこの累積膜は分子認識機能を有するため、特定分子との吸着、反応触媒作用等によって分子配列の立体構造に変化が生じ、これに伴って二次高

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調波発生機能が変化するので、この変化を検知して前記特定分子の濃度を測定することができる。前記分子認識機能を有する化合物と、前記二次高調波を発生する化合物とは、それぞれ異なる単分子膜成膜成分中に存在してもよく、この場合には２つの単分子膜を交互に重ねることで非対称構造の累積膜を容易に形成することができる。

【０００９】前記二次高調波を発生する化合物は、電子供与基及び電子受容基に挟まれた発色団を有する化合物とすることによって二次高調波発生能を強化することができる。また前記単分子膜が親水基と疎水基とを有する化合物を含むものであれば前記ＬＢ法によって容易に累積膜を形成することができる。

【００１０】また本発明による分子認識機能膜は、前記のように特定分子の影響による二次高調波の変化を検知してこの特定分子の濃度を測定することが可能なためセンサーとして使用することができる。本発明のセンサーの構成は、一般的に行われているメーカーフリンジ法又はウエッジ法を転用すればよく（「光学的測定ハンドブック」朝倉書店　IV-1.2.1. 494頁）、基本波を発生して分子認識機能膜へ入射する発光素子と、ここから発生する二次高調波を受光する受光素子と、この受光素子からの信号を処理する信号処理部とからなり、上記分子認識機能膜から発生する測定試料に固有の二次高調波発生能を測定してその濃度を計測することができる。

【００１１】

【作用】本発明の分子認識機能膜は、特定化合物との吸着、反応触媒作用等によって分子配列の立体構造に変化が生じ、これに伴って二次高調波発生機能も変化するため、この変化を検知して特定化合物の濃度を測定することができる。

【００１２】

【実施例】以下に本発明の実施例を説明する。図１は本発明に基づくセンサーを示す概念図である。分子認識機能膜１は透明基板２上に形成され、透明なフローセル３内の流路４を流れる測定試料５が直接分子認識機能膜１に触れるように設置されている。発光素子６から発せられた光（基本波）は、フローセル３及び測定試料５の流れる流路４を透過して分子認識機能膜１を照射する。分子認識機能膜１を透過した二次高調波を含む光は、カットフィルター７で二次高調波以外の波長をカットされて受光素子８へ入射する。こうして検出した二次高調波の＊

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＊変化を信号処理部９で処理して測定試料５中に含まれる特定試料の濃度を算出する。

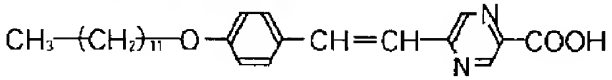
【００１３】図２乃至図５は本発明の分子認識機能膜１及びその構成分子を表したものである。図２(a)の分子認識機能膜１は分子１０及び分子１１からなる単分子膜Ａと、分子１０及び分子１２からなる単分子膜Ｂとを後述のヘテロＹ型構造の形式に重ねたものである。これらの単分子膜はＬＢ法によって容易に累積膜とすることができる。同図中では２層のみ表示してあるが、実際には２層に限定されるものではない。

【００１４】分子１０及び１１は図３に示す構造を有している。即ち分子１０は、図３(a)にあるように疎水基１３及び親水基１４からなり、また分子１１は疎水基１５、親水基１６及び発色団１７らなる。分子１１内の疎水基１５は電子供与基を、また親水基１６は電子受容基を兼用しているが、勿論、疎水基と電子供与基、親水基と電子受容基は別々の基であってもよい。また分子１０に用いる疎水基１３及び親水基１４は公知のどのような基でもよいが、分子１１との親和性を考慮してそれぞれ電子供与基を兼ねる疎水基１５及び電子受容基を兼ねる親水基１６と同じまたは同種のものを使用することが好ましい。電子供与基を兼ねる疎水基１５としてはアルキル基又はアリール基が好ましく、電子受容基を兼ねる親水基１６としては－ＣＯＯＭ基、－SO_3Ｍ基等が好ましい（Ｍは水素原子又はアルカリ金属原子若しくはアルカリ土類金属原子を表す）。

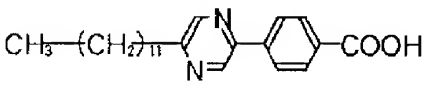
【００１５】また極性の発色団１７としてはニトロ基、アゾ基、共役ジエン等公知のものを利用することができるが、この発色団１７は電子供与基と電子授与基とに挟まれていることが好ましい。以上から、分子１０としてはアラキジン酸、ステアリン酸バリウム、ステアリルアルコール、ステアリルメルカプタン、ステアリルアミン等の化合物が挙げられ、また分子１１としては例えば（化１）乃至（化５）に示すような化合物が挙げられる。また分子１２は特定分子を識別してその作用を受ける機能を有する化合物である。分子１２としては、例えば特定分子を吸着する化合物として、特定の外形を有するイオンだけを吸着する機能を有するクラウンエーテルが挙げられ、また特定の基質のみの反応を触媒する酵素等も挙げられる。

【００１６】

【化１】

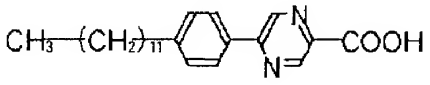


【化２】



【化３】

【化４】



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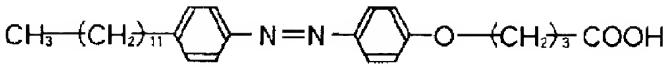
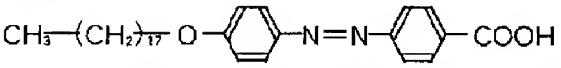


【化５】



【００１７】図４はＬＢ法による単分子膜の累積の型を示す模式図である。本図中、(b)は親水基同士（また図には表されていないが、疎水基同士も）が接触した対称型の構造（Ｙ型構造）をとっており安定な膜である。しかしこの累積膜は構造が対称であるため膜全体としては分極せず、従って二次高調波発生能力が劣る。一方、(a)（Ｘ型構造）及び(c)（Ｚ型構造）は累積された単分子膜内の分子が全て一方向に向いている非対称構造である。従って、分子内の電子供与基と電子受容基の効果によって膜全体が分極し、二次高調波発生を効率よく行うことができる。しかし(b)の対称型構造と違って親水基と疎水基が接触しているため、かなり不安定な膜である。一方(d)の構造は、２種の単分子膜を交互に累積したものであって、各種類の膜単位で見ると、分子が全て一方向に向いている非対称構造をとっている（ヘテロＹ型構造）。このヘテロＹ型構造は、親水基同士（また図には表されていないが、疎水基同士も）が接触した構造であるため安定な膜であり、本発明に係る非対称構造として最も望ましいものである。

【００１８】上記ヘテロＹ型構造の累積膜を形成する場合、例えば図２(a)のように形成する場合を説明すると、単分子膜Ａは疎水基及び親水基を有する分子１０と電子供与基、電子受容基及び発色団を有する分子１１とからなる。従って、単分子膜Ａは二次高調波発生機能を有する膜となる。また単分子膜Ｂは疎水基及び親水基を有する分子１０と特定分子を識別してその作用を受ける機能を有する分子１２とからなる。即ち単分子膜Ｂは分子認識機能膜となる。この単分子膜Ｂの上に更に単分子膜Ａ、単分子膜Ｂ、単分子膜Ａ、単分子膜Ｂ、……と重ねて累積膜を形成するが、複数の単分子膜Ａ内の分子は全て親水基（電子受容基）を上方に向けており、また複数の単分子膜Ｂ内の分子は親水基を全て下方に向けている（分子１２が酵素の場合には酵素は親水性であることが多いため、分子１０の親水基の側に並ぶ。また分子１２が疎水性の場合はアルキル基等を付加することによって、同じく親水基の側に並べることができる）。従って膜全体として見ると非対称構造の膜となり、単分子膜Ａについては丁度平面電池を直列につないだ形となって膜上面が－に、膜下面が＋に分極し、二次高調波発生を効率よく行うことができる。

【００１９】上記の膜をセンサーとして使用した場合、そのメカニズムは明確ではないが、分子１２内の分子認識機能を有する化合物は特定分子の作用によって膨張、歪み等の変形を生じ、これが単分子膜Ａの発色団含有分子１１に伝達されて分子１１の方向性等に影響を与えるものと思われる。いずれにしても特定分子の作用によって固有の二次高調波発生機能に変化が生じ、しかもこの変化は特定分子の濃度と相関があるため、これを利用して特定分子の濃度を測定することが可能となる。

【００２０】図２(b)は１種類の単分子膜が二次高調波発生機能及び分子認識機能を併せ持つ例である。分子認識機能を有する分子１２が親水性である場合は、この図のように親水基及び疎水基を持つ分子１０によって単分子膜内に固定することができる。ＬＢ法による累積膜を形成するには、この１種類の単分子膜を重ねる方法もあるが、もう１種類の例えば分子１０のような親水基及び疎水基を持つ分子からなる単分子膜を使用して、前記ヘテロＹ型の構造をとるほうが形成も容易であり安定性も良い。

【００２１】図２(c)は１種類の膜が二次高調波発生機能及び分子認識機能を併せ持つ例である。この膜は分子認識機能を有する分子１２が疎水性であるため、単分子膜を水面に形成したときに膜の疎水基側に乗り上げた形となっている。この膜もＬＢ法を用いて容易に累積膜とすることができる。またこの場合も上記(b)の例と同じく、もう１種類の例えば分子１０のような親水基及び疎水基を持つ分子からなる単分子膜を使用して、前記ヘテロＹ型の構造とするほうが形成も容易であり安定性も良い。

【００２２】上記のような、２種類の膜を交互に重ねるＬＢ法による累積膜は、例えば図５に示すような二槽式ラングミュアトラフ１８を用いて形成することができる。二槽式ラングミュアトラフ１８には単分子膜を形成するための槽（トラフ）Ｉ及び槽（トラフ）IIが設けられている。槽Ｉ及び槽IIには水が張られておりそれぞれの水面には例えば図２(a)の単分子膜Ａ及びＢの各組成物が有機溶媒に溶解されて滴下されている。この有機溶剤は揮発して各単分子膜の成分のみが気体膜として展開される。こうして形成された各単分子膜を図示しないバリアで圧縮して所望の表面圧で圧縮して凝縮膜とする。

【００２３】上記のように槽Ｉ及び槽IIに単分子膜が形成された状態で透明基板２の膜が形成される部分を図のように槽Ｉに沈める。このとき単分子膜Ａが透明基板２へ移動しても表面圧が変化しないようにバリアを移動させる。本発明に用いる透明基板２は透明性があって試料或いは溶剤等で劣化しないものであればよく、例えばガラス板等の無機材料やアクリル板等の有機ポリマーによって形成されたもの等が挙げられる。次に透明基板２を沈めた状態のままフレキシブル・ゲート１９ａ、１９ｂを通過させて槽IIへ移動する。槽IIへ移動させた透明基板２は仮想線で示してある。なおフレキシブル・ゲート１９ａ、１９ｂの間の槽IIIは２種類の単分子膜の混濁を防ぐため設けられた中間槽である。槽IIへ移した透明

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基板２の膜形成部分を引き上げることによって単分子膜Ａの上に単分子膜Ｂが重なる。今度は透明基板２を引き上げた状態のままフレキシブル・ゲート１９ｂ、１９ａを通過させて槽Ｉに戻し、膜形成部分を沈める。以上の繰り返しによって累積膜を得ることができる。

【００２４】以下に本発明の具体的な実施例を説明する。

実施例１

透明基板２として、屈折率nd＝1.523の透明ガラス板を洗浄して用いた。図５に示した二槽式ラングミュアトラフ１８を使用し、槽Ｉには（化１）の化合物（図３の分子１０に属する、発色団を含む化合物）及びアラキジン酸（図３の分子１０に属する疎水基及び親水基を有する化合物。$CH_3(CH_2)_{18}COOH$）をクロロホルムに溶解した溶液を滴下して単分子膜Ａを形成し、表面圧25mN/mに圧縮した。一方槽IIには、酵素であるグルコースオキシダーゼ（図２の分子１２に属する、分子認識機能を有する化合物）及び酵素固定化剤ＤＰＰＥ（図３の分子１０に属する、疎水基及び親水基を有する化合物。Dipalmitoylphosphatidylethanolamine　シグマ社製）をクロロホルムに溶解した溶液を滴下して単分子膜Ｂを形成し、表面圧8mN/mに圧縮した。これら酵素及び酵素固定化剤は上記の他公知の種々のものを使用することができる。

【００２５】次に上述の累積膜形成方法に従って、透明基板２上に単分子膜Ａ及びＢが交互に合計８１層重ねられたヘテロＹ型構造のＬＢ膜を形成した。その後、透明基板２の片面のみをクロロホルムにさらして形成された膜の片面を除去した。この透明基板２上に形成された膜を用いて、先ず未処理のまま二次高調波強度を測定し、次に純水に膜面を５分間さらしてから測定し、最後に100mg/ml濃度のグルコース水溶液に膜面を５分間さらしてから測定した。この結果、二次高調波強度は未処理の場合及び純水で処理した場合はほぼ同じ強度を示したが、グルコース水溶液と接触させた場合には明らかな強度低下を示した。なお、二次高調波強度の測定は、ＹＡＧレーザーの基本波（λ＝1.064μm）を用いて、上記膜を形成した透明基板２に膜側から照射し、透過光中の基本波を赤外線カットフィルターで除去して検出した。

【００２６】実施例２

先ず透明基板２として屈折率nd＝1.523の透明ガラス板を２枚準備し、うち１枚の片面には真空蒸着法によりＣr膜（2nm）を形成しておいた。図５に示した二槽式ラングミュアトラフ１０を使用し、槽Ｉには（化２）の化合物（図３の分子１１に属する発色団を含む化合物）、クラウンエーテル（図２の分子１２に属する分子認識機能を有する化合物。Bis(12Crown4)）及びアラキジン酸（図３の分子１０に属する疎水基及び親水基を有する化合物。$CH_3(CH_2)_{18}COOH$）をクロロホルムに溶解した溶液を滴下して単分子膜Ａを形成し、表面圧23mN/m

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に圧縮した。一方槽IIには（化２）の化合物を（化３）の化合物に変えた以外は槽Ｉの組成と同じ組成物を用い、クロロホルムに溶解してこの溶液を滴下し、単分子膜Ｂを形成した。表面圧は同じく23mN/mに調整した。上記クラウンエーテルは上記の他公知の種々のものを使用することができるが、本例のような２環式のものは、特定のイオンを吸着した際にクラウンエーテルの構造にねじれが生じて膜構造の物理的変化が著しくなるため、二次高調波強度の変化も大きくなるので好ましい。

【００２７】次に先ず片面にＣr膜を形成しておいたガラス透明基板２を槽IIに下降し、槽Ｉで上昇し、この操作を１０回繰返してヘテロＹ型膜を20層形成した。その後、Ｃr膜を形成しておいた面とは反対の面をクロロホルムにさらしてこの面に形成された膜を除去した。一方Ｃr膜を形成していないガラス透明基板２についてはまず槽Ｉで下降し、槽IIで上昇し、この操作を１０回繰返してヘテロＹ型膜を19層形成した。その後、片面をクロロホルムにさらして形成された片面の膜を除去した。そしてこの２つの基板の、膜を除去した面同士を貼り合せて本発明に基づく両面に膜を設けた基板を完成した。

【００２８】次に実施例１と同じＹＡＧレーザーを用いて、上記基板を回転させながら（即ち入射角を変えながら）発生する二次高調波強度を測定し、図６に示すフリンジパターンを得た。このようなパターンが生ずる理由は、それぞれの膜で発生した二次高調波が互いに干渉するためであり、入射角によって変化するのは行路長が変化するためである。そこで二次高調波が極小値をとる角度に上記基板を固定し、純水に５分間さらした後に二次高調波強度を測定したところ変化はなかった。次に2mol/l及び10mol/l濃度の塩化ナトリウム水溶液に５分間さらした後に測定したところ、濃度変化に相関性を持って明らかな二次高調波強度の増加が見られた。

【００２９】比較例１

単分子膜Ｂの形成用組成物中にグルコースオキシダーゼを添加しない以外は実施例１と同様にして累積膜をガラス透明基板２上に形成し、二次高調波強度を測定したところ、100mg/ml濃度のグルコース水溶液で膜面を処理した場合にも強度変化は生じなかった。

【００３０】比較例２

単分子膜Ａの形成用組成物中に（化１）の化合物を添加しない以外は実施例１と同様にして累積膜をガラス透明基板２上に形成し、二次高調波強度を測定したところ、未処理、純水処理及び100mg/ml濃度のグルコース水溶液処理のいずれの場合も二次高調波は検出できなかった。

【００３１】

【発明の効果】以上に説明した如く本発明によれば、特定化合物の吸着機能、この化合物との錯体形成機能、或いはこの化合物の酵素反応、免疫反応等の触媒作用等、多種の作用を二次高調波発生強度の変化に転換して検出することができるため、特定分子の濃度を容易に検出す

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ることができる。

【図面の簡単な説明】

【図１】本発明に基づくセンサーを示す概略図

【図２】本発明に係る分子認識機能膜の一部を模式的に示した図

【図３】本発明に係る分子認識機能膜を構成する成分分子の一部を模式的に示した図

【図４】本発明に係る分子認識機能膜のＬＢ法による累積型式を模式的に示した図

【図５】二槽式ラングミュアトラフの概略図

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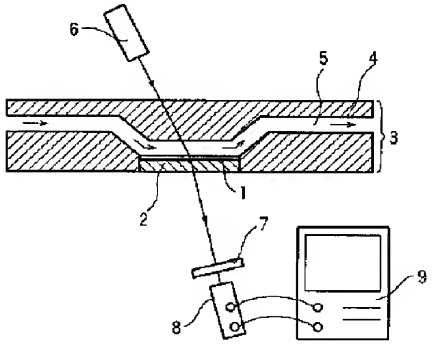
【図６】基本波入射角と発生した二次高調波強度との相関図

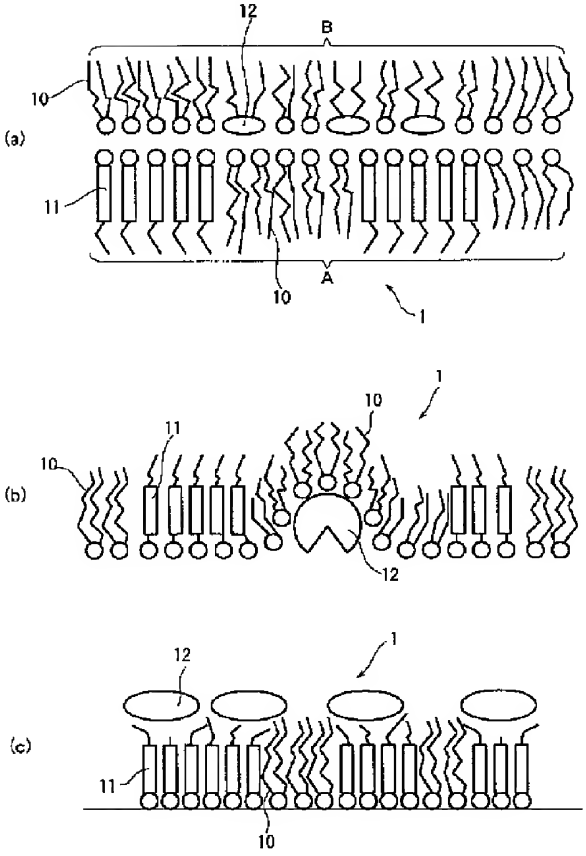
【符号の説明】

１…分子認識機能膜、２…透明基板、３…フローセル、４…流路、５…測定試料、６…発光素子、７…カットフィルター、８…受光素子、９…信号処理部、１０、１１、１２…分子、１３…疎水基、１４…親水基、１５…電子供与基を兼ねる疎水基、１６…電子受容基を兼ねる親水基、１７…発色団、１８…二槽式ラングミュアトラフ、１９ａ、１９ｂ…フレキシブル・ゲート。

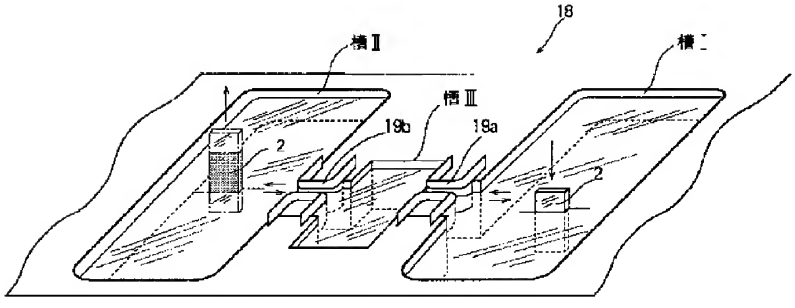
【図１】



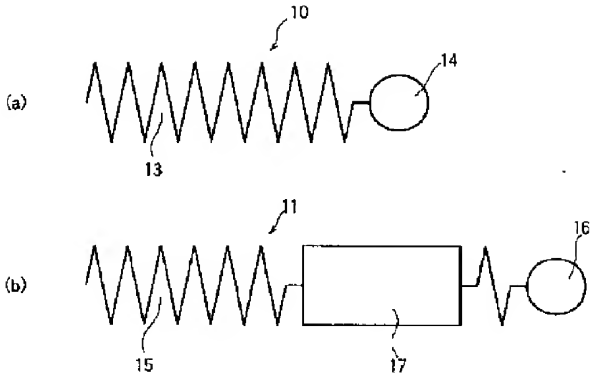
【図２】



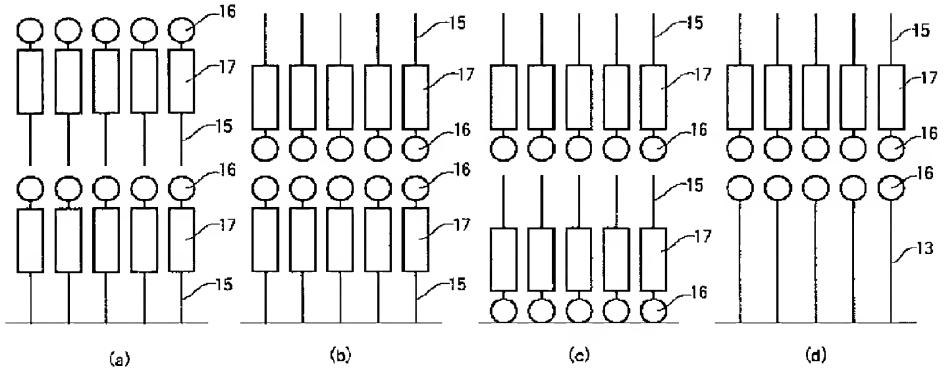
【図５】



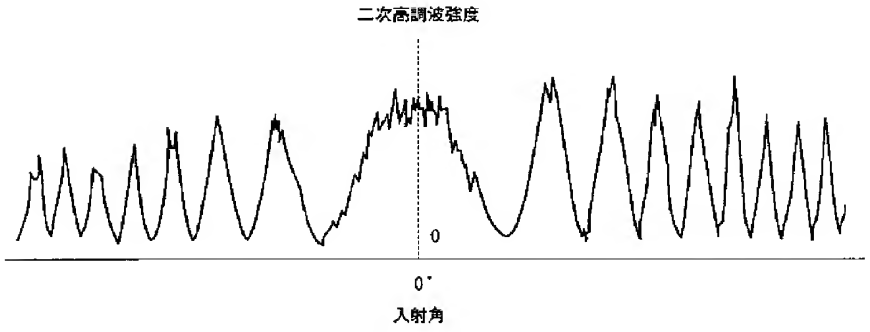
【図３】



【図４】



【図６】



B6

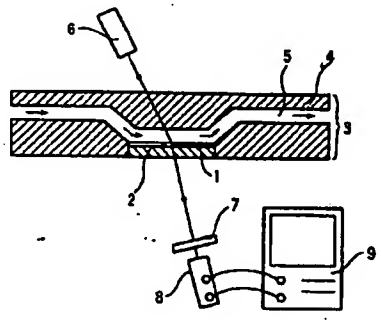
(Abstract)

(Object)

To put forward molecular recognition function membrane which detects the concentration change of specific molecule by converting into change of second harmonic generation intensity, and a sensor using the same.

Construction

A molecular recognition function membrane 1 is formed on a transparent substrate 2, and measurement sample 5 passing through passage 4 in the transparent flow cell 3 is established so as to directly come into contact with the molecular recognition function membrane 1. The light emitted from the light emitting element 6 (fundamental wave) transmits the flow cell 3 and the passage 4 that flows the measurement sample 5, and irradiates the molecular recognition function membrane 1. The light that transmitted the molecular recognition function membrane 1 including the second harmonic wave is filtered for the wavelength other than second harmonic wave using a cut filter 7, and is emitted to optical receptor element 8. The change of second harmonic waves detected is processed by signal processing part 9, and the concentration of the specific sample contained in the measurement sample 5 is calculated.



Patent Claims

Claim 1

A molecular recognition function membrane of the kind wherein a molecular recognition substance having a catalytic function such as enzyme reaction or the like with specific compound, an adsorption function with the said compound or a complex formation function with the said compound is immobilised on a carrier,

characterised in that the aforesaid membrane is the accumulation layer in which monolayers

are accumulated in asymmetric structure, and a compound which generates second harmonic wave is contained in at least one of the plurality of monolayers that construct the said accumulation layer.

Claim 2

A molecular recognition function membrane in accordance with Claim 1, wherein the aforesaid compound which generates the second harmonic waves is a compound having a chromophore placed in between electron donor group and electron accept group.

Claim 3

A molecular recognition function membrane in accordance with Claim 1, wherein the aforesaid molecular recognition function membrane is the accumulation layer formed by accumulating at least one monolayer including the compound having hydrophilic group and hydrophobic group.

Claim 4

A sensor comprising

a molecular recognition function membrane containing a compound which generates second harmonic waves in at least one of the plurality of monolayers which construct the accumulation layer formed by accumulating monolayers in asymmetric structure,

a light emitting element which generates fundamental wave and emits to the aforesaid molecular recognition function membrane,

an optical receptor element which detects the second harmonic waves generated there, and

a signal processing part that processes the signal from the optical receptor element.

Detailed Description of the Invention

(0001)

Sphere of Application in Industry

This invention relates to the following, namely, a molecular recognition function membrane which carries out concentration measurement of organic and inorganic compounds or biological compounds or the like by second harmonic generation, and a sensor using the said membrane.

(0002)

Technology of the Prior Art

The living body has a capability to recognise inorganic and organic substances with high

selectivity. Biosensors have been known which detect a sample in sample solution quickly and simply using functional molecules having such high selectivity, or cells, tissues or even the living body itself as the substance selective functioning part.

(0003)

The function of aforesaid sensor can be regarded by dividing into a part that selectively recognises the specific sample and causes reaction, and a part that senses the change of conductivity, heat generation, luminescence or the like of sample due to the reaction thereof and converts into signal. As biopolymers which recognize the sample and react therewith, proteins species such as enzymes, antibodies, receptors or the like are known, and these are used by dispersing and immobilising in the membrane made of natural polymer or synthetic polymer. On the other hand, as the part that senses the reaction and converts into signal, electrodes such as oxygen electrode, hydrogen peroxide electrode, ion electrode, gas electrode or the like are generally used. Moreover, recently a number sensors and the like using luminescence of the aforesaid sample have been proposed.

(0004)

The electrochemical measurement method using the aforesaid electrode has defects such that (1) electrical and magnetic noises are readily generated during measurement, (2) measurement of trace sample cannot be made due to the use of reference electrode, or the like. Whereas, optical measurement method, which is disclosed in for example Kokai 61-292045, Kokai 63-75542, Kokai 63-271162, has advantages such that (1) high sensitive light detection became possible due to technological innovation of recent years, (2) electrical and magnetic noises are hardly generated during measurement, (3) reference electrode is not necessary, and the like, and furthermore, when an optic fibre is used for the incidence and detection of light, there are characteristics or the like that the reagent exchange in reaction field becomes easy as physical contact between the reaction field and the optic fibre is not necessary, moreover, there is a possibility that plurality of samples can be simultaneously measured using plurality of wavelengths.

(0005)

Meanwhile, organic non-linear optical materials have been studied energetically as material for the aforesaid second harmonic generation, because of the possibility of realizing, in principle, higher nonlinear optical characteristics than inorganic material. In order to realise high second harmonic generation (SHG), molecules with high molecular polarity is needed to be arranged in sequential structure which does not have center of inversion (asymmetric

orientation). Therefore, the study which had been proceeding originally on single crystal material has been carried out recently on accumulation layer having asymmetric orientation structure formed by Langmuir-Blodgett (LB) method (Japan Society of Applied Physics Vol. 60, issue 6 (1991) P586). This LB method has characteristics or the like such that (1) sequence structure which does not have center of inversion (asymmetric orientation) can be easily formed, (2) the thin membrane always having optical axis in the normal direction of substrate is obtained, (3) the thin membrane can be controlled in molecular level, (4) patterning is possible using lithography technique by introducing polymerisable group, (5) refractive index can be controlled by introducing substance such as heavy metal or the like, and moreover, when a molecule with high molecular polarisation is used as film-forming component, there is a great feature that the formed LB membrane itself also shows high polarisation characteristics, and accordingly displaying high second harmonic generation.

(0006)

The aforesaid second harmonic generation is one of characteristics of non-linear optical material, but where a change occurs on the molecular arrangement or molecular structure of LB membrane, the non-linear optical characteristics are also affected. There is an example of applying this principle in which protonation of LB membrane film-forming component was carried out with hydrogen chloride gas and the change in second harmonic during this was analysed (The Electrochemical Society, spring meeting 1991, 2A22).

(0007)

Problems to be Overcome by this Invention

Aforesaid LB method of the prior art can generate second harmonic waves, but how to utilise this as a sensor is still in a research stage, and is not applied in practical use. Even in the study linking the aforesaid change of molecular structure and non-linear optical characteristics, this technique was unsuitable to use as analysis apparatus because the LB membrane constituting molecule receives direct chemical change.

(0008)

Means to Overcome these Problems

In order to overcome the aforesaid problems, this invention forms accumulation layer by introducing a compound having molecular recognition function in a film-forming component of monolayer and a compound that generates second harmonic waves. Accordingly, because this accumulation layer has molecular recognition function, the tertiary structure of molecular arrangement is changed due to adsorption of specific

molecule, reaction catalytic action and the like, and the second harmonic generation function is changed accompanied by this, therefore the concentration of the said specific molecule can be measured by detecting this change. The aforesaid compound having the molecular recognition function and the compound that generates the second harmonic wave may be respectively present in different monolayer film-forming components, and in this case, accumulation layer of asymmetric structure can be easily formed by alternating accumulation of two monolayers.

(0009)

The second harmonic generation property can be intensified by forming the aforesaid compound that generates the second harmonic waves into a compound having chromophore placed in between electron donor group and electron accept group. Moreover, if the aforesaid monolayer includes the compound having hydrophilic group and hydrophobic group, accumulation layer can be readily formed by the aforesaid LB method.

(0010)

Moreover, molecular recognition function membrane in accordance with this invention can measure the concentration of specific molecule by detecting the change of second harmonic waves due to the effect of the specific molecule as described above, and therefore can be used as a sensor. As the structure of a sensor of this invention, generally used Maker-fringe method or wedge method may be followed ("Optical Measurement Handbook" Asakura bookstore IV-1.2.1. p.494), and the sensor comprises a luminescence element which generates fundamental wave and emits to the molecular recognition function membrane, an optical receptor element which detects second harmonic waves generated from the luminescence element and a signal processing part that processes the signal from this optical receptor element, and the concentration can be measured by measuring the second harmonics inherent to the measuring sample generated from the aforesaid molecular recognition function membrane.

(0011)

Action

Because the tertiary structure of molecular arrangement is altered due to adsorption of specific compound, reaction catalytic action or the like, accompanied with this, the second harmonic generation function is also changed, thereby the molecular recognition function membrane of this invention can measure the concentration of specific compound by detecting this change.

(0012)

Examples

Below, Examples of this invention are described. Figure 1 is a schematic diagram to illustrate a sensor on the basis of this invention. A molecular recognition function membrane 1 is formed on a transparent substrate 2, and a measurement sample 5 passing through the passage 4 in the transparent flow cell 3 is established so as to directly come into contact with the molecular recognition function membrane 1. The light emitted from the light emitting element 6 (fundamental wave) transmits the flow cell 3 and the passage 4 flowing the measurement sample 5 and irradiates the molecular recognition function membrane 1. The light that transmitted the molecular recognition function membrane 1 including the second harmonic wave is filtered for the wavelength other than second harmonic wave using a cut filter 7, and is emitted to optical receptor element 8. The change of second harmonic waves detected in this way is processed in signal processing part 9, and the concentration of the specific sample contained in the measurement sample 5 is calculated.

(0013)

Figure 2 to Figure 5 show molecular recognition function membrane 1 of this invention and constituting molecules thereof. The molecular recognition function membrane 1 of Figure 2(a) is a membrane wherein monolayer A formed from molecule 10 and molecule 11 and monolayer B formed from molecule 10 and molecule 12 are accumulated in a form of hetero Y type structure described later. These monolayers can be readily made into the accumulation layer by LB method. Only two layers are illustrated in the Figures, but the form s not limited to two layers in practice.

(0014)

Molecules 10 and 11 have structures shown in Figure 3. In other words, the molecule 10 comprises hydrophobic group 13 and hydrophilic group 14 as shown in Figure 3 (a), and moreover the molecule 11 comprises hydrophobic group 15, hydrophilic group 16 and chromophore 17. The hydrophobic group 15 in the molecule 11 also acts as electron donor group and the hydrophilic group 16 also acts as electron accept group, however, of course the hydrophobic group and the electron donor group, the hydrophilic group and the electron accept group may be separate group. Moreover, the hydrophobic group 13 and the hydrophilic group 14 to be used in the molecule 10 may be any kind of well known groups, however, on consideration of the affinity to the molecule 11, respectively the same one or

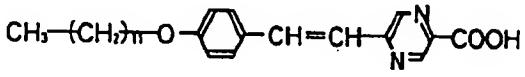
the same kind of hydrophobic group 15 also acting as electron donor group and the hydrophilic group 16 also acting as electron accept group are preferably used. As hydrophobic group 15 also acting as electron donor group, alkyl group or aryl group is preferred, and as the hydrophilic group 16 also acting as electron accept group, -COOM group, $-SO_3M$ group and the like are preferred (M denotes a hydrogen atom or alkaline earth metal atom or alkali metal atom).

(0015)

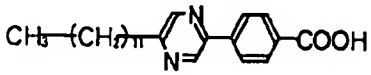
Moreover, as polar chromophore 17, well known species such as nitro group, azo group, conjugated diene and the like can be used, and the said chromophore 17 is preferably placed in between the electron donor group and the electron accept group. From the above, the compounds such as arachic acid, barium stearate, stearyl alcohol, stearyl mercaptan, stearylamine and the like are nominated as the molecule 10 and moreover, as the molecule 11, the compounds shown for example in Formula 1 to Formula 5 may be nominated. Moreover, the molecule 12 is a compound having function to distinguish the specific molecule and to receive action thereof. As the molecule 12, for example as the compound that adsorbs specific molecule, Crown ether having function to adsorb only the ion having specific contour may be proposed. Enzymes that only catalyses reaction of specific substrate may be proposed, too.

(0016)

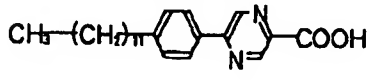
Compound 1



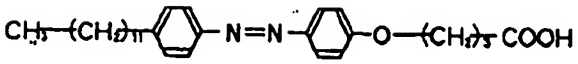
Compound 2



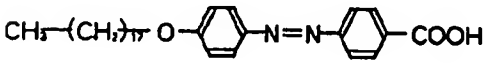
Compound 3



Compound 4



Compound 5



(0017)

Figure 4 is a schematic diagram illustrating a type of accumulation of monolayer by LB method. In the Figure, (b) has a symmetric type structure (Y type structure) in which hydrophilic groups form contact to each other (moreover, although not shown in Figure, hydrophobic groups also form contact to each other), and is a stable membrane. However, because this accumulation layer is symmetric, the membrane as a whole is not polarized, and accordingly, the second harmonic generation capability is inferior. On the other hand, (a) (X type structure) and (c) (Z type structure) are asymmetric structures in which all the molecules in accumulated monolayer are facing one direction. Accordingly, the whole membrane is polarized due to the effect of electron donor group and electron accept group in the molecule, and the second harmonic generation can be carried out efficiently. However, unlike the symmetric structure of (b), because the hydrophilic group and the hydrophobic group form a contact, it is a rather unstable membrane. On the other hand, as for the structure of (d), two kinds of monolayers are accumulated in alternation, and by looking at as membrane unit of each species, it has asymmetric structure in which all the molecules are facing one direction (hetero Y type structure). Because this hetero Y type structure is the structure in which hydrophilic groups form contact to each other (moreover, although not shown in Figure, hydrophobic groups also form contact to each other), and is a stable membrane, and it is the most desirable form as the asymmetric structure in accordance with this invention.

(0018)

When accumulation layer of the aforesaid hetero Y type structure is to be formed, for example a case of forming the layer as in Figure 2 (a) is described. Monolayer A comprises from the molecule 10 having hydrophobic group and hydrophilic group and molecule 11 having electron donor group, electron accept group and chromophore. Accordingly, the monolayer A is a membrane having second harmonic generation function. Moreover, monolayer B comprises the molecule 10 having hydrophobic group and hydrophilic group

and molecule 12 having function to distinguish specific molecule and to receive action thereof. In other words, the monolayer B is a molecular recognition function membrane. Accumulation layer is formed by further accumulating monolayer A, monolayer B, monolayer A, monolayer B, ... on this monolayer B, however, all the hydrophilic groups (electron accepter groups) are facing upward in the molecules in plurality of monolayer A, and all the hydrophilic groups are facing downward in the molecules in plurality of monolayer B (when molecule 12 is an enzyme, because enzymes are often hydrophilic, it align on the side of hydrophilic group of molecule 10, and moreover, when the molecule 12 is hydrophobic, it can be aligned on the side of hydrophilic group in the same way by addition of alkyl group or the like). Accordingly, looking as membrane as a whole, it becomes membrane of asymmetric structure, and the monolayer A becomes a form as if plane batteries are connected in series, polarization occurs into – at the membrane upper part and + at the membrane lower part, and second harmonic generation can be carried out efficiently.

(0019)

When the aforesaid membrane is used as sensor, although the mechanism thereof is not clear, the compound having molecular recognition function in the molecule 12 generates deformation such as expansion, distortion or the like due to the action of specific molecule, and it is thought that this deformation is communicated to chromophore-containing molecule 11 of monolayer A, and affects directional property or the like of molecule 11. In any case, the change occurs in the second harmonic generation function inherent to the action of specific molecule, and besides, this change has a correlation to the concentration of specific molecule, therefore the concentration of specific molecule can be measured using this.

(0020)

Figure 2(b) is an example of one kind of monolayer having both the second harmonic generation function and the molecular recognition function. When molecule 12 having molecular recognition function is hydrophilic, it can be immobilised in the monolayer by molecule 10 having hydrophilic group and hydrophobic group as seen in this Figure. When forming accumulation layer by LB method, there is a method to accumulate this one kind of monolayer, but formation is easier and stability is better when a structure of said hetero Y type is formed using for example monolayer formed from the molecule having hydrophilic group and hydrophobic group such as molecule 10.

(0021)

Figure 2(c) is an example of one kind of membrane having both the second harmonic generation function and the molecular recognition function. Because molecule 12 having molecular recognition function is hydrophobic, this membrane is in a form of being carried over to the hydrophobic group side of membrane when the monolayer is formed on the water surface. This membrane can be readily formed into the accumulation layer using LB method, too. Moreover, in this case, in the same way as in the example of the aforesaid (b), formation is easier and stability is better when a structure of said hetero Y type is formed using for example monolayer formed from the molecule having hydrophilic group and hydrophobic group such as molecule 10.

(0022)

The aforesaid accumulation layer using LB method wherein two kinds of membranes are accumulated alternatively can be formed using for example two-trough system Langmuir trough 18 as shown in Figure 5. Trough I and trough II for forming monolayer are established in two-trough system Langmuir trough 18. The trough I and trough II are filled with water, and to each water surface is added dropwise each composition of monolayer A and B of for example Figure 2(a) dissolved in organic solvent. This organic solvent is volatilised, and only the component of each monolayer is developed as gaseous membrane. Each monolayer formed in this way is compressed with the barrier which is not illustrated, and condensed film is made by compressing at a desired surface pressure.

(0023)

A part where the membrane of transparent substrate 2 is formed is immersed in trough I as shown in Figure in a state in which the monolayers have been formed in trough I and trough II as described above. During this procedure, barrier is moved so that the surface pressure does not change even when the monolayer A is moved to the transparent substrate 2. Any substrate having transparency which is not deteriorated by sample or solvent may be used as transparent substrate 2 in this invention, and for example substrate formed from inorganic material such as glass plate or the like and organic polymer such as acryl plate or the like may be proposed. Next, transparent substrate 2 is moved to trough II by passing through flexible gate 19a, 19b while being immersed state. The transparent substrate 2 moved to the trough II is shown with imaginary line. Moreover, the trough III established between flexible gate 19a and 19b is an intermediate tank provided in order to prevent mixing of two kinds of monolayers. The monolayer B is accumulated on monolayer A by pulling up the membrane forming part of the transparent substrate 2 transferred to the trough II. Then, the

transparent substrate 2 is returned to the trough I by passing through flexible gate 19b, 19a while in the elevated state, and then the membrane forming part is immersed. Accumulation layer can be obtained by repeating the above procedures.

(0024)
Embodying Examples of this invention are described below.

Example 1
As transparent substrate 2, transparent glass plate of refractive index nd= 1.523 was washed and used. Using two-trough system Langmuir trough 18 showed in Figure 5, a chloroform solution of compound of Formula 1 (the compound including chromophore belonging to molecule 10 of Figure 3) and arachidic acid (the compound having hydrophobic group and hydrophilic group belonging to molecule 10 of Figure 3, CH3(CH2)18COOH) was added dropwise in trough I to form monolayer A, and it was compressed at a surface pressure of 25 mN/m. On the other hand, a chloroform solution of an enzyme, glucose oxidase (the compound having molecular recognition function belonging to molecule 12 of Figure 2) and enzyme immobilising agent DPPE (the compound having hydrophobic group and hydrophilic group belonging to molecule 10 of Figure 3 Dipalmitoyl phosphatidyl ethanolamine, Sigma Corp.) was added dropwise in trough II to form monolayer B, and it was compressed at a surface pressure of 8 mN/m. As for these enzyme and enzyme immobilising agent, well known various kinds can be used other than above-mentioned species.

(0025)
Thereafter, according to the aforesaid accumulation layer forming procedure, hetero Y type structured LB membrane was formed in which monolayers A and B were accumulated alternatively in total of 81 layers on the transparent substrate 2. Thereafter, one side of the formed membrane was eliminated by exposing only one side of transparent substrate 2 to chloroform. Using membrane formed on this transparent substrate 2, firstly, the second harmonic intensity was measured in untreated state, then the measurement was carried out after exposing the membrane surface to pure water for five minutes, and finally the measurement was carried out after exposing the membrane surface to glucose aqueous solution of 100 mg/ml concentration for five minutes. As a result, the untreated second harmonic waves and the second harmonic wave treated with pure water showed almost same intensity, but the second harmonic waves contacted with glucose aqueous solution showed apparent lowering of the intensity. Moreover, the measurement of second harmonic

intensity was carried out as follows. The transparent substrate 2 on which the aforesaid membrane was formed was irradiated from the membrane side using fundamental wave (λ = 1.064 μm) of YAG laser, and detection was carried out by eliminating the fundamental wave in the transmitted beam using infrared radiation cut filter.

(0026)

Example 2

Firstly, two pieces of transparent glass plates with refractive index nd= 1.523 were prepared as transparent substrate 2, and Cr membrane (2 nm) was formed beforehand by vacuum deposition method on one side of one of them. Using two-trough system Langmuir trough 10 showed in Figure 5, a chloroform solution of compound of Formula 2 (the compound including chromophore belonging to molecule 11 of Figure 3), crown ether (the compound having molecular recognition function belonging to molecule 12 of Figure 2, Bis(12Crown4)) and arachidic acid (the compound having hydrophobic group and hydrophilic group belonging to molecule 10 of Figure 3, CH3(CH2)18COOH) was added dropwise in trough I to form monolayer A, and it was compressed at a surface pressure of 23 mN/m. On the other hand, a chloroform solution of the same composition used in trough I except using the compound of Formula 3 instead of the compound of Formula 2was added dropwise in trough II, and monolayer B was formed. The surface pressure was adjusted to 23 mN/m in the same way. Various well known species can be used as the aforesaid crown ether other than above-mentioned, but the bicyclic system of this example is preferable, because when a specific ion is adsorbed, torsion is generated in the structure of crown ether, and physical change of membrane structure becomes markedly, and therefore the change of secondary harmonic intensity is also large.

(0027)

Thereafter, firstly, the glass transparent substrate 2 on which Cr membrane had been formed on one side beforehand was immersed in trough II and lifted in trough I, and this procedure was repeated ten times, and 20 layers of hetero Y type membrane was formed. Thereafter, the side opposite to the side on which Cr membrane was formed was exposed to chloroform, and the membrane formed on this side was eliminated. On the other hand, the glass transparent substrate 2 on which Cr membrane had not been formed was firstly immersed in trough I and then lifted in trough II, and this procedure was repeated ten times, and 19 layers of hetero Y type membranes were formed. Thereafter, one side of the formed membrane was eliminated by exposing one side to chloroform. Next, the sides of these two substrates from which membranes had been eliminated were laminated to each other, and

the substrate provided with membrane on both sides on the basis of this invention was completed.

(0028)

Thereafter, using the same YAG laser as in Example 1, generated second harmonic intensity was measured while rotating the aforesaid substrate (in other words, while changing the angle of incidence), and fringe pattern shown in Figure 6 was obtained. The reason why such pattern was generated is that the second harmonic waves generated in each membrane interfere with each other, and the reason why it changed according to the angle of incidence is due to the change of path length. Therefore, the aforesaid substrate was fixed at the angle wherein the second harmonic became the minimum value, and it was exposed to pure water for five minutes, and thereafter the second harmonic intensity was measured, as a result, no change was observed. Thereafter, the measurement was carried out after exposing to sodium chloride aqueous solution in concentrations of 2 mol/l and 10 mol/l for five minutes, as a result, marked increase of the second harmonic intensity in correlation with concentration change was observed.

(0029)

Comparative Example 1

Accumulation layer was formed on glass transparent substrate 2 in the same way as in Example 1, except that glucose oxidase was not added to the composition for formation of monolayer B, and the second harmonic intensity was measured. As a result, the change in intensity did not occur even when the membrane surface was treated with glucose aqueous solution of 100 mg/ml concentration.

(0030)

Comparative Example 2

Accumulation layer was formed on glass transparent substrate 2 in the same way as in Example 1, except that the compound of Formula 1 was not added to the composition for formation of monolayer A, and the second harmonic intensity was measured. As a result, the second harmonic wave could not be detected in any case of untreated, pure water treated and treatment with glucose aqueous solution 100 mg/ml concentration.

(0031)

Advantages Afforded by this Invention

As described above, in accordance with this invention, the concentration of specific

molecule can be readily detected, because various actions such as adsorption function of specific compound, complex forming function with this compound or catalytic action such as enzyme reaction, immunoreaction or the like of the said compound can be detected by converting to the change in the second harmonic generation intensity.

Brief Description of the Figures

Figure 1

A schematic diagram showing a sensor on the basis of this invention.

Figure 2

A schematic figure showing a part of molecular recognition function membrane in accordance with this invention.

Figure 3

A schematic figure showing a part of component molecule constituting the molecular recognition function membrane in accordance with this invention.

Figure 4

A schematic figure showing accumulation forms by LB method of molecular recognition function membrane in accordance with this invention.

Figure 5

A schematic diagram of two-trough system Langmuir trough.

Figure 6

A correlation diagram of the angle of incidence of the fundamental wave and the generated second harmonic intensity.

Key to Symbols

1... molecular recognition function membrane, 2... transparent substrate, 3... flow cell, 4... passage, 5... element sample, 6... light emitting element, 7... cut filter, 8... optical receptor element, 9... signal processing part, 10,11,12... molecules, 13... hydrophobic group, 14... hydrophilic group, 15... hydrophobic group also acting as electron donor group, 16... hydrophilic group also acting as electron accept group, 17... chromophore, 18... two-trough system Langmuir trough, 19... flexible gate.

Figure 1

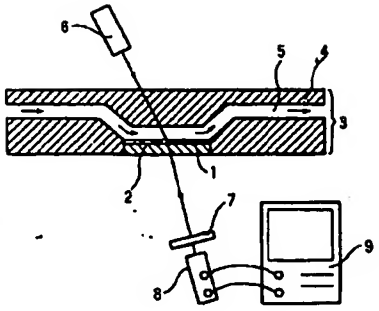


Figure 2

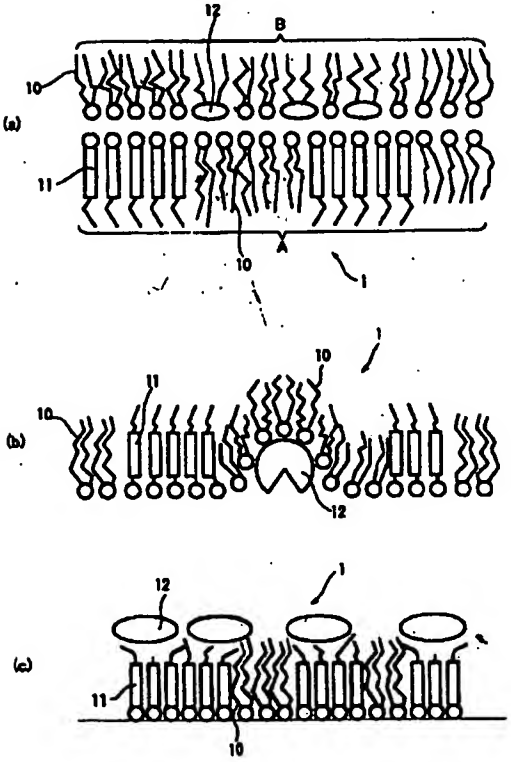


Figure 3

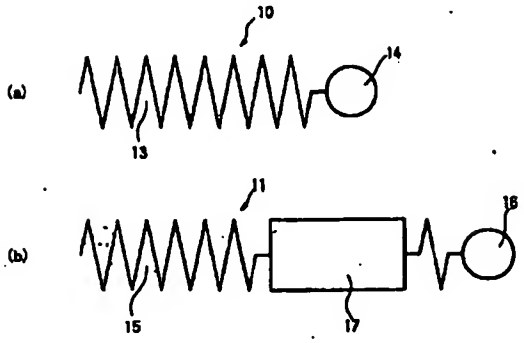


Figure 4

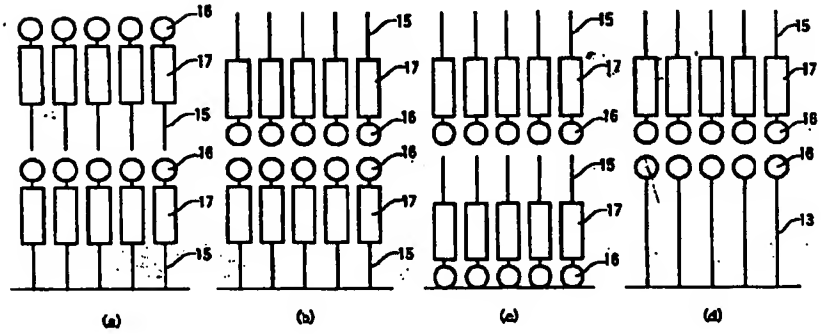


Figure 5

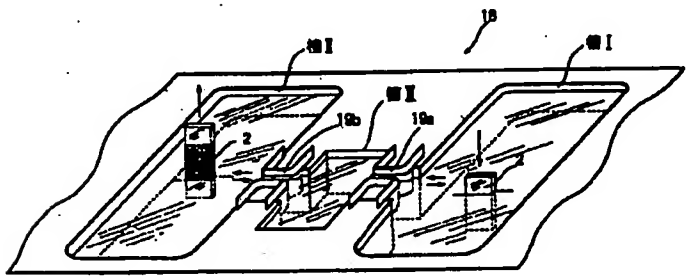
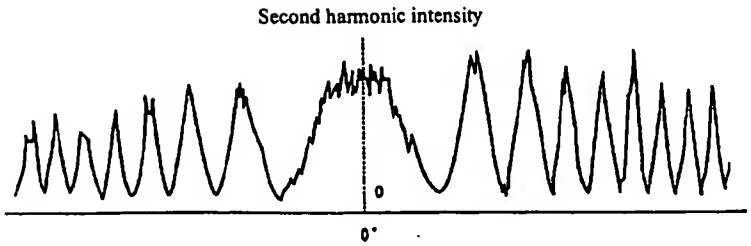


Figure 6



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